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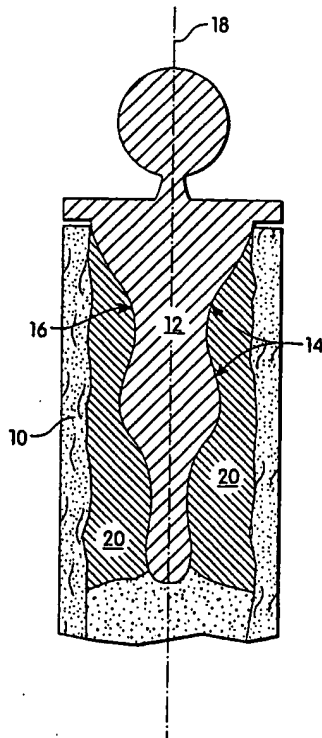
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(54) Title: PROSTHETIC DEVICES HAVING ENHANCED OSTEOGENIC PROPERTIES



(57) Abstract

A prosthetic device comprising a prosthesis coated with substantially pure osteogenic protein is disclosed. A method for biologically fixing prosthetic devices *in vivo* is also disclosed. In this method, a prosthesis is implanted in an individual in contact with a substantially pure osteogenic protein, enhancing the strength of the bond between the prosthesis and the existing bone at the joining site.

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PROSTHETIC DEVICES HAVING ENHANCED
OSTEOGENIC PROPERTIES

Reference to Related Applications

This application is a continuation-in-part of copending U.S. application Serial No. 07/841,646, filed 2/21/92, which is a continuation-in-part of U.S. Application Serial Nos. :

- 1) 07/827,052, filed January 28, 1992, a divisional of USSN 07/179,406, filed April 8, 1988, now US 4,968,590;
- 2) 07/579,865, filed September 7, 1990, a divisional of USSN 07/179,406;
- 3) 07/621,849, filed December 4, 1990, a divisional of USSN 07/232,630, filed August 15, 1988, now abandoned, that was a continuation-in-part of 07/179,406;
- 4) 07/621,988, filed December 4, 1990, a divisional of 07/315,342 filed February 23, 1989, now US 5,011,691 and which is a continuation-in-part of 07/232,630;
- 5) 07/810,560, filed December 20, 1991, a continuation of 07/660,162, filed February 22, 1991, now abandoned, that was a continuation of 07/422,699, filed October 17, 1989, now abandoned, that was a continuation-in-part of 07/315,342;
- 6) 07/569,920, filed August 20, 1990, now abandoned, that was a continuation-in-part of 07/422,699 and 07/483,913, which is continuation-in-part of 07/422,613, filed October 17, 1989, now US 4,975,526 and which is a continuation-in-part of 07/315,342;
- 7) 07/600,024, filed October 18, 1990, a continuation-in-part of 07/569,920;
- 8) 07/599,543, filed October 18, 1990, a continuation-in-part of 07/569,920;
- 9) 07/616,374, filed November 21, 1990, a divisional of 07/422,613; and
- 10) 07/483,913, filed February 22, 1990.

- 2 -

Background of the Invention

Regeneration of skeletal tissues is thought to be regulated by specific protein factors that are naturally present within bone matrix. When a bone is damaged, these factors stimulate cells to form new cartilage and bone tissue which replaces or repairs lost or damaged bone. Regeneration of bone is particularly important where prosthetic implants are used without bonding cement to replace diseased bone, as in hip replacement. In these cases, formation of a tight bond between the prosthesis and the existing bone is very important, and successful function depends on the interaction between the implant and the bone tissue at the interface.

Bone healing can be stimulated by one or more osteogenic proteins which can induce a developmental cascade of cellular events resulting in endochondral bone formation. Proteins stimulating bone growth have been referred to in the literature as bone morphogenic proteins, bone inductive proteins, osteogenic proteins, osteogenin or osteoinductive proteins.

U.S. 4,968,590 (November 6, 1990) discloses the purification of "substantially pure" osteogenic protein from bone, capable of inducing endochondral bone formation in a mammal when implanted in the mammal in association with a matrix, and having a half maximum activity of at least about 25 to 50 nanograms per 25 milligrams of implanted matrix. Higher activity subsequently has been shown for this protein, e.g., 0.8-1.0 ng of osteogenic protein per mg of implant matrix, as disclosed in U.S. Patent 5,011,691. This patent also disclosed a consensus DNA sequence probe useful for identifying genes encoding osteogenic proteins, and a number of human genes encoding osteogenic proteins identified using the consensus probe, including a previously unidentified gene referred to therein as "OP1" (osteogenic protein-1). The consensus probe also identified DNA

- 3 -

sequences corresponding to sequences termed BMP-2 Class I and Class II ("BMP2" and "BMP4" respectively) and BMP3 in International Appl. No. PCT/US87/01537. The osteogenic proteins encoded by these sequences are referred to herein as "CBMP2A," "CBMP2B", and "CBMP3", respectively. U.S. 5,011,691 also defined a consensus "active region" required for osteogenic activity and described several novel biosynthetic constructs using this consensus sequence which were capable of inducing cartilage or bone formation in a mammal in association with a matrix.

These and other researchers have stated that successful implantation of the osteogenic factors for endochondral bone formation requires that the proteins be associated with a suitable carrier material or matrix which maintains the proteins at the site of application. Bone collagen particles which remain after demineralization, guanidine extraction and delipidation of pulverized bone have been used for this purpose. Many osteoinductive proteins are useful cross-species. However, demineralized, delipidated, guanidine-extracted xenogenic collagen matrices typically have inhibited bone induction in vivo. Sampath and Reddi (1983) Proc. Natl. Acad. Sci. USA, 80: 6591-6594. Recently, however, Sampath et al. have described a method for treating demineralized guanidine-extracted bone powder to create a matrix useful for xenogenic implants. See, U.S. 4,975,526 (December 4, 1990). Other useful matrix materials include for example, collagen; homopolymers or copolymers of glycolic acid, lactic acid, and butyric acid, including derivatives thereof; and ceramics, such as hydroxyapatite, tricalcium phosphate and other calcium phosphates. Combinations of these matrix materials also may be useful.

Orthopedic implants have traditionally been attached to natural bone using bone cement. More recently, cementless prostheses have been used, in which the portion of the prosthesis that contacts the natural bone is coated with a

- 4 -

porous material. M. Spector, J. Arthroplasty, 2(2):163-176 (1987); and Cook et al., Clin. Orthoped. and Rel. Res., 232: 225-243 (1988). Cementless fixation is preferred because biological fixation of the prosthesis is stronger when osseointegration is achieved. The porous coatings reportedly stimulate bone ingrowth resulting in enhanced biological fixation of the prosthesis. However, there are several problems with porous-coated prostheses. For example, careful prosthetic selection is required to obtain a close fit with the bone to ensure initial mechanical stabilization of the device, and surgical precision is required to ensure initial implant-bone contact to promote bone ingrowth. Porous coated implants have not resulted in bone ingrowth in some instances, for example, in porous coated tibial plateaus used in knee replacements. A prosthetic implant that results in significant bone ingrowth and forms a strong bond with the natural bone at the site of the join would be very valuable.

The current state of the art for the anchoring of embedded implants such as dental implants also is unsatisfactory. Typically, dental implant fixation first requires preparing a tooth socket in the jawbone of an individual for prosthesis implantation by allowing bone ingrowth into the socket void to fill in the socket. This preparatory step alone can take several months to complete. The prosthesis then is threaded into the new bone in the socket and new bone is allowed to regrow around the threaded portion of the implant embedded in the socket. The interval between tooth extraction and prosthetic restoration therefore can take up to eight months. In addition, threading the prosthesis into bone can damage the integrity of the bone. Prosthetic dental implants that can improve osseointegration and reduce the time and effort for fixation would be advantageous.

Summary of the Invention

The present invention relates to a method of enhancing the growth of bone at the site of implantation of a prosthesis to form a bond between the prosthesis and the existing bone. As used herein, a prosthesis is understood to describe the addition of an artificial part to supply a defect in the body. The method involves coating or otherwise contacting all or a portion of the prosthesis that will be in contact with bone with a substantially pure osteogenic protein. The prosthesis first may be coated with the osteogenic protein and then implanted in the individual at a site wherein the bone tissue and the surface of the prosthesis are maintained in close proximity for a time sufficient to permit enhanced bone tissue growth between the tissue and the implanted prosthesis. Alternatively, the site of implantation first may be treated with substantially pure osteogenic protein and the prosthesis then implanted at the treated site such that all or a portion of the prosthesis is in contact with the osteogenic protein at the site, and the prosthesis, the osteogenic protein and the existing bone tissue are maintained in close proximity to one another for a time sufficient to permit enhanced bone tissue growth between the tissue and the prosthesis. The osteogenic protein associated with the implanted prosthesis stimulates bone growth around the prosthesis and causes a stronger bond to form between the prosthesis and the existing bone than would form between the prosthesis and the bone in the absence of the protein.

In a preferred embodiment of the present method a prosthetic device, such as an artificial hip replacement device, e.g., a metallic device made from titanium, for example, is first coated with an osteogenic material which induces bone ingrowth. When the device is subsequently implanted into the individual, bone growth around the site of the implant is enhanced, causing a strong bond to form

- 6 -

between the implant and the existing bone. The present method results in enhanced biological fixation of the prosthesis in the body, which is particularly important for weight bearing prostheses. Prostheses defining a microporous surface structure are locked in place as bone formation occurs within the micropores. The metal or ceramic prosthesis may itself define such a structure, or the prosthesis may be coated to provide an adherent porous surface. Materials useful for this purpose include, for example, collagen, homopolymers of glycolic acid, lactic acid, and butyric acid, including derivatives thereof; and ceramics such as hydroxyapatite, tricalcium phosphate or other calcium phosphates. Combinations of these materials may be used. A substantially pure osteogenic protein is then bound to the uncoated or coated prosthesis. Alternatively, the osteogenic protein can be mixed with the coating material, and the mixture adhered onto the surface of the prosthesis.

In another embodiment of the present invention, osteogenic protein combined with a matrix material is packed into an orifice prepared to receive the prosthetic implant. The surface of the implant also may be coated with osteogenic protein, as described above. The implant has a shape defining one or more indentations to permit bone ingrowth. The indentations are preferably transverse to the longitudinal axis of the implant. In general, the longitudinal axis of the implant will be parallel to the longitudinal axis of the bone which has been treated to receive the implant. New bone grows into the indentations thereby filling them, integrates with the surface of the implant as described above, and integrates with existing bone. Thus, the prosthesis can be more tightly fixed into the orifice, and "latched" or held in place by bone growing into the indentations, and by osseointegration of new bone with the surface of the implant, both of which are stimulated by the osteogenic protein.

- 7 -

In a specific embodiment, a dental implant is used to replace missing teeth. The implant typically comprises a threaded portion which is fixed into the jawbone and a tooth portion configured to integrate with the rest of the patient's teeth. The implant is coated with osteogenic protein (with or without a matrix or carrier) and threaded or screwed into a tooth socket in the jawbone prepared to receive it (e.g., bone has been allowed to grow into and fill the socket void.) In a particularly preferred embodiment, the socket is prepared to receive the implant by packing the void with a bone growth composition composed of osteogenic protein dispersed in a suitable carrier material. The combination of osteogenic protein and carrier is referred to herein as an "osteogenic device." The osteogenic protein promotes osseointegration of the implant into the jawbone without first requiring bone growth to fill the socket, and without requiring that the prosthesis be threaded into existing bone, which may weaken the integrity of the the existing bone. Accordingly, the time interval between tooth extraction and prosthetic restoration is reduced significantly. It is anticipated that prosthetic restoration may be complete in as little time as one month. In addition, the ability of the osteogenic protein to promote osseointegration of the prosthesis will provide a superior anchor.

A prosthetic device coated with the above osteogenic protein also is the subject of the present invention. All or a portion of the device may be coated with the protein. Generally, only the portion of the device which will be in contact with the existing bone will be coated.

The present method and device results in enhanced biological fixation of the prosthesis. A strong bond is formed between the existing bone and the prosthesis, resulting in improved mechanical strength at the joining

- 8 -

site. Higher attachment strength means that the prosthesis will be more secure and permanent, and therefore will be more comfortable and durable for the patient.

- 9 -

Brief Description of the Drawing

The sole Figure of the drawing schematically depicts a cross-sectional view of a portion of a prosthesis implanted in a femur and illustrates the latching action of bone ingrowth in accordance with an embodiment of the invention.

Detailed Description of the Invention

The present invention relates to a method for enhancing osseointegration between a prosthesis and natural bone in an individual at the site of implantation of the prosthesis. The method involves providing a prosthesis to a site of implantation together with substantially pure osteogenic protein such that the osteogenic protein is in contact with all or a portion of the implanted prosthesis. The protein promotes osseointegration of the prosthesis and the bone, resulting in a strong bond having improved tensile strength.

Osteogenic proteins which are useful in the present invention are substantially pure osteogenically active dimeric proteins. As used herein "substantially pure" means substantially free of other contaminating proteins having no endochondral bone formation activity. The protein can be either natural-sourced protein derived from mammalian bone or recombinantly produced proteins, including biosynthetic constructs. The natural-sourced proteins are characterized by having a half maximum activity of at least 25 to 50 ng per 25 mg of demineralized protein extracted bone powder, as compared to rat demineralized bone powder.

The natural-sourced osteogenic protein in its mature, native form is a glycosylated dimer having an apparent molecular weight of about 30 kDa as determined by SDS-PAGE. When reduced, the 30 kDa protein gives rise to two glycosylated peptide subunits having apparent molecular weights of about 16 kDa and 18 kDa. In the reduced state, the protein has no detectable osteogenic activity. The unglycosylated protein, which also has osteogenic activity, has an apparent molecular weight of about 27 kDa. When reduced, the 27 kDa protein gives rise to two unglycosylated polypeptides having molecular weights of about 14 kDa to 16 kDa. The recombinantly-produced osteogenic protein describes a class of dimeric proteins capable of inducing endochondral bone formation in a mammal comprising a pair of

- 11 -

polypeptide chains, each of which has an amino acid sequence sufficiently duplicative of the sequence of the biosynthetic constructs or COP-5 Or COP-7, (SEQ. ID NOS.3 and 4), such that said pair of polypeptide chains, when disulfide bonded to produce a dimeric species is capable of inducing endochondral bone formation in a mammal. As defined herein, "sufficiently duplicative" is understood to describe the class of proteins having endochondral bone activity as dimeric proteins implanted in a mammal in association with a matrix, each of the subunits having at least 60% amino acid sequence homology in the C-terminal cysteine-rich region with the sequence of OPS (residues 335 to 431, SEQ. ID No. 1). "Homology" is defined herein as amino acid sequence identity or conservative amino acid changes within the sequence, as defined by Dayoff, et al., Atlas of Protein Sequence and Structure; vol.5, Supp.3, pp.345-362, (M.O. Dayoff, ed. Nat'l Biomed. Research Fdn., Washington, D.C., 1979.) Useful sequences include those comprising the C-terminal sequences of DPP (from *Drosophila*), Vgl (from *Xenopus*), Vgr-1 (from mouse), the OP1 and OP2 proteins, the CBMP2, CBMP3, and CBMP4 proteins (see U.S. Pat. No. 5,011,691 and U.S. Application Serial No. 07/841,646 by Oppermann et al., filed February 21, 1992, the disclosures of both of which are hereby incorporated by reference, as well as the proteins referred to as BMP5 and BMP6 (see WO90/11366, PCT/US90/01630.) A number of these proteins also are described in WO88/00205, U.S. Patent No. 5,013,649 and WO91/18098. Table I provides a list of the preferred members of this family of osteogenic proteins.

TABLE I - OSTEOGENIC PROTEIN SEQUENCES

hOP1	-	DNA sequence encoding human OP1 protein (Seq. ID No. 1 or 3). Also referred to in related applications as "OP1", "hOP-1" and "OP-1".
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- 12 -

- OP1 - Refers generically to the family of osteogenically active proteins produced by expression of part or all of the hOP1 gene. Also referred to in related applications as "OPI" and OP-1".
- hOP1-PP - Amino acid sequence of human OP1 protein (prepro form), Seq. ID No. 1, residues 1-431. Also referred to in related applications as "OP1-PP" and "OPP".
- OP1-18Ser - Amino acid sequence of mature human OP1 protein, Seq. ID No. 1, residues 293-431. N-terminal amino acid is serine. Originally identified as migrating at 18 kDa on SDS-PAGE in COS cells. Depending on protein glycosylation pattern in different host cells, also migrates at 23kDa, 19kDa and 17kDa on SDS-PAGE. Also referred to in related applications as "OP1-18".
- OPS - Human OP1 protein species defining the conserved 6 cysteine skeleton in the active region (97 amino acids, Seq. ID No. 1, residues 335-431). "S" stands for "short".
- OP7 - Human OP1 protein species defining the conserved 7 cysteine skeleton in the active region (102 amino acids, Seq. ID No. 1, residues 330-431).
- OP1-16Ser - N-terminally truncated mature human OP1 protein species. (Seq. ID No. 1, residues 300-431). N-terminal amino acid is serine; protein migrates at 16kDa or 15kDa on

- 13 -

SDS-PAGE, depending on glycosylation pattern. Also referred to in related applications as "OP-16S".

- OP1-16Leu - N-terminally truncated mature human OP1 protein species, Seq. ID No. 1, residues 313-431. N-terminal amino acid is leucine; protein migrates at 16 or 15kDa on SDS-PAGE, depending on glycosylation pattern. Also referred to in related applications as "OP-16L".
- OP1-16Met - N-terminally truncated mature human OP1 protein species, Seq. ID No. 1, residues 315-431. N-terminal amino acid is methionine; protein migrates at 16 or 15kDa on SDS-PAGE, depending on glycosylation pattern. Also referred to in related applications as "OP-16M".
- OP1-16Ala - N-terminally truncated mature human OP1 protein species, Seq. ID No. 1, residues 316-431. N-terminal amino acid is alanine, protein migrates at 16 or 15 kDa on SDS-PAGE, depending on glycosylation pattern. Also referred to in related applications as "OP-16A".
- OP1-16Val - N-terminally truncated mature human OP1 protein species, Seq. ID No. 1, residues 318-431. N-terminal amino acid is valine; protein migrates at 16 or 15 kDa on SDS-PAGE, depending on glycosylation pattern. Also referred to in related applications as "OP-16V".

- 14 -

- mOP1 - DNA encoding mouse OP1 protein, Seq. ID No. 8. Also referred to in related applications as "mOP-1".
- mOP1-PP - Prepro form of mouse protein, Seq. ID No. 8, residues 1-430. Also referred to in related applications as "mOP-1-PP".
- mOP1-Ser - Mature mouse OP1 protein species (Seq. ID No. 8, residues 292-430). N-terminal amino acid is serine. Also referred to in related applications as "mOP1" and "mOP-1".
- mOP2 - DNA encoding mouse OP2 protein, Seq. ID No. 12. Also referred to in related applications as "mOP-2".
- mOP2-PP - Prepro form of mOP2 protein, Seq. ID No. 12, residues 1-399. Also referred to in related applications as "mOP-2-PP".
- mOP2-Ala - Mature mouse OP2 protein, Seq. ID No. 12, residues 261-399. N-terminal amino acid is alanine. Also referred to in related applications as "mOP2" and "mOP-2".
- hOP2 - DNA encoding human OP2 protein, Seq. ID No. 10. Also referred to in related applications as "hOP-2".
- hOP2-PP - Prepro form of human OP2 protein, Seq. ID No. 10, res. 1-402). Also referred to in related applications as "hOP-2-PP".

- 15 -

- hOP2-Ala - Possible mature human OP2 protein species:
Seq. ID No. 10, residues 264-402. Also
referred to in related applications as
"hOP-2".
- hOP2-Pro - Possible mature human OP2 protein species:
Seq. ID No. 10, residues 267-402. N-terminal
amino acid is proline. Also referred to in
related applications as "hOP-2P".
- hOP2-Arg - Possible mature human OP2 protein species:
Seq. ID No. 10, res. 270-402. N-terminal
amino acid is arginine. Also referred to in
related applications as "hOP-2R".
- hOP2-Ser - Possible mature human OP2 protein species:
Seq. ID No. 10, res. 243-402. N-terminal
amino acid is serine. Also referred to in
related applications as "hOP-2S".
- Vgr-1-fx C-terminal 102 amino acid residues of the
murine "Vgr-1" protein (Seq. ID No. 7).
- CBMP2A C-terminal 101 amino acid residues of the
human BMP2A protein. (Residues 296-396 of
Seq. ID No. 14).
- CBMP2B C-terminal 101 amino acid residues of the
human BMP2B protein. (Seq. ID No. 18).
- BMP3 Mature human BMP3 (partial sequence, Seq. ID
No. 16. See U.S. 5,011,691 for C-terminal 102
residues, "CBMP3.")
- BMP5-fx C-terminal 102 amino acid residues of the
human BMP5 protein. (Seq ID No. 20).

- 16 -

BMP6-fx	C-terminal 102 amino acid residues of the human BMP6 protein. (Seq ID No. 21).
COP5	Biosynthetic osteogenic 96 amino acid sequence (Seq. ID No. 3).
COP7	Biosynthetic osteogenic 96 amino acid sequence (Seq. ID No. 4).
DPP-fx	C-terminal 102 amino acid residues of the Drosophila "DPP" protein (Seq. ID No. 5).
Vgl-fx	C-terminal 102 amino acid residues of the Xenopus "Vgl" protein (Seq. ID No. 6).

The members of this family of proteins share a conserved six or seven cysteine skeleton in this region (e.g., the linear arrangement of these C-terminal cysteine residues is conserved in the different proteins.) See, for example, OPS, whose sequence defines the six cysteine skeleton, or OP7, a longer form of OP1, comprising 102 amino acids and whose sequence defines the seven cysteine skeleton.) In addition, the OP2 proteins contain an additional cysteine residue within this region.

This family of proteins includes longer forms of a given protein, as well as species and allelic variants and biosynthetic mutants, including addition and deletion mutants and variants, such as those which may alter the conserved C-terminal cysteine skeleton, provided that the alteration still allows the protein to form a dimeric species having a conformation capable of inducing bone formation in a mammal when implanted in the mammal in association with a matrix. In addition, the osteogenic proteins useful in devices of this invention may include forms having varying glycosylation patterns and varying

- 17 -

N-termini, may be naturally occurring or biosynthetically derived, and may be produced by expression of recombinant DNA in procaryotic or eucaryotic host cells. The proteins are active as a single species (e.g., as homodimers), or combined as a mixed species.

A particularly preferred embodiment of the proteins useful in the prosthetic devices of this invention includes proteins whose amino acid sequence in the cysteine-rich C-terminal domain has greater than 60% identity, and preferably greater than 65% identity with the amino acid sequence of OPS.

In another preferred aspect, the invention comprises osteogenic proteins comprising species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX" which accommodates the homologies between the various identified species of the osteogenic OP1 and OP2 proteins, and which is described by the amino acid sequence of Sequence ID No. 22.

In still another preferred aspect, the invention comprises nucleic acids and the osteogenically active polypeptide chains encoded by these nucleic acids which hybridize to DNA or RNA sequences encoding the active region of OP1 or OP2 under stringent hybridization conditions. As used herein, stringent hybridization conditions are defined as hybridization in 40% formamide, 5 X SSPE, 5 X Denhardt's Solution, and 0.1% SDS at 37°C overnight, and washing in 0.1 X SSPE, 0.1% SDS at 50°C.

The invention further comprises nucleic acids and the osteogenically active polypeptide chains encoded by these nucleic acids which hybridize to the "pro" region of the OP1 or OP2 proteins under stringent hybridization conditions. As used herein, "osteogenically active polypeptide chains" is understood to mean those polypeptide chains which, when dimerized, produce a protein species having a conformation such that the pair of polypeptide chains is capable of

- 18 -

inducing endochondral bone formation in a mammal when implanted in a mammal in association with a matrix or carrier.

Given the foregoing amino acid and DNA sequence information, the level of skill in the art, and the disclosures of U.S. Patent 5,011,691 and published PCT specification US 89/01469, published October 19, 1989, the disclosures of which are incorporated herein by reference, various DNAs can be constructed which encode at least the active domain of an osteogenic protein useful in the devices of this invention, and various analogs thereof (including species and allelic variants and those containing genetically engineered mutations), as well as fusion proteins, truncated forms of the mature proteins, deletion and addition mutants, and similar constructs. Moreover, DNA hybridization probes can be constructed from fragments of any of these proteins, or designed de novo from the generic sequence. These probes then can be used to screen different genomic and cDNA libraries to identify additional osteogenic proteins useful in the prosthetic devices of this invention.

The DNAs can be produced by those skilled in the art using well known DNA manipulation techniques involving genomic and cDNA isolation, construction of synthetic DNA from synthesized oligonucleotides, and cassette mutagenesis techniques. 15-100mer oligonucleotides may be synthesized on a DNA synthesizer, and purified by polyacrylamide gel electrophoresis (PAGE) in Tris-Borate-EDTA buffer. The DNA then may be electroeluted from the gel. Overlapping oligomers may be phosphorylated by T4 polynucleotide kinase and ligated into larger blocks which may also be purified by PAGE.

The DNA from appropriately identified clones then can be isolated, subcloned (preferably into an expression vector), and sequenced. Plasmids containing sequences of interest then can be transfected into an appropriate host cell for

- 19 -

protein expression and further characterization. The host may be a procaryotic or eucaryotic cell since the former's inability to glycosylate protein will not destroy the protein's morphogenic activity. Useful host cells include E. coli, Saccharomyces, the insect/baculovirus cell system, myeloma cells, CHO cells and various other mammalian cells. The vectors additionally may encode various sequences to promote correct expression of the recombinant protein, including transcription promoter and termination sequences, enhancer sequences, preferred ribosome binding site sequences, preferred mRNA leader sequences, preferred signal sequences for protein secretion, and the like.

The DNA sequence encoding the gene of interest also may be manipulated to remove potentially inhibiting sequences or to minimize unwanted secondary structure formation. The recombinant osteogenic protein also may be expressed as a fusion protein. After being translated, the protein may be purified from the cells themselves or recovered from the culture medium. All biologically active protein forms comprise dimeric species joined by disulfide bonds or otherwise associated, produced by folding and oxidizing one or more of the various recombinant polypeptide chains within an appropriate eucaryotic cell or in vitro after expression of individual subunits. A detailed description of osteogenic proteins expressed from recombinant DNA in E. coli is disclosed in U.S. Serial No. 422,699 filed October 17, 1989, the disclosure of which is incorporated herein by reference. A detailed description of osteogenic proteins expressed from recombinant DNA in numerous different mammalian cells is disclosed in U.S. Serial No. 569,920 filed August 20, 1990, the disclosure of which is hereby incorporated by reference.

Alternatively, osteogenic polypeptide chains can be synthesized chemically using conventional peptide synthesis techniques well known to those having ordinary skill in the

- 20 -

art. For example, the proteins may be synthesized intact or in parts on a solid phase peptide synthesizer, using standard operating procedures. Completed chains then are deprotected and purified by HPLC (high pressure liquid chromatography). If the protein is synthesized in parts, the parts may be peptide bonded using standard methodologies to form the intact protein. In general, the manner in which the osteogenic proteins are made can be conventional and does not form a part of this invention.

The osteogenic proteins useful in the present invention are proteins which, when implanted in a mammalian body, induce the developmental cascade of endochondral bone formation including recruitment and proliferation of mesenchymal cells, differentiation of progenitor cells, cartilage formation, calcification of cartilage, vascular invasion, bone formation, remodeling and bone marrow differentiation. The osteogenic protein in contact with the present prostheses can induce the full developmental cascade of endochondral bone formation at the site of implantation essentially as it occurs in natural bone healing.

Prostheses which can be used with the present method include porous or non-porous orthopedic prostheses of the types well known in the art. Such prostheses are generally fabricated from rigid materials such as metals, including for example, stainless steel, titanium, molybdenum, cobalt, chromium and/or alloys or oxides of these metals. Such oxides typically comprise a thin, stable, adherent metal oxide surface coating. The prostheses are preferably formed from or coated with porous metals to permit infiltration of the bone, but non-porous materials also can be used. Porous metallic materials for use in prostheses are described, for example, by Spector in J. Arthroplasty, 2(2):163-176 (1987), and by Cook et al. in Clin. Orthoped. and Rel. Res., 232:225-243 (1988), the teachings of both of which are hereby incorporated herein by reference. Metallic

- 21 -

prostheses may be used for major bone or joint replacement and for repairing non-union fractures, for example, where the existing bone has been destroyed by disease or injury.

In a preferred embodiment of the present device and method, the prosthesis is coated with a material which enhances bone ingrowth and fixation, in addition to the protein. Materials which are useful for this purpose are biocompatible, and preferably in vivo biodegradable and non-immunogenic. Such materials include, for example, collagen, hydroxyapatite, homopolymers or copolymers of glycolic acid lactic acid, and butyric acid and derivatives thereof, tricalcium phosphate or other calcium phosphates, metal oxides, (e.g., titanium oxide), and demineralized, guanidine extracted bone.

The present coated prostheses are prepared by applying a solution of the protein, and optionally, hydroxylapatite or other material to all or a portion of the prosthesis. The protein can be applied by any convenient method, for example, by dipping, brushing, immersing, spraying or freeze-drying. Hydroxylapatite is preferably applied by a plasma spraying process. The protein is preferably applied by immersing the prostheses in a solution of the protein under conditions appropriate to induce binding or precipitation of the protein from solution onto the implant. The amount of protein which is applied to the implant should be a concentration sufficient to induce endochondral bone formation when the prosthesis is implanted in the recipient. Generally a concentration in the range of at least $5\mu\text{g}$ protein per 3.4cm^2 surface area is sufficient for this purpose. If hydroxylapatite or other carrier material is used, it is applied to the prosthesis in an amount required to form a coating of from about 15μ to about 60μ thick. A layer about 25μ thick of hydroxylapatite has been used to improve implant fixation, as shown in the exemplification.

- 22 -

In one aspect, the prosthesis comprises a device configured for insertion into an orifice prepared to receive the prosthesis. In this embodiment, as illustrated in the Figure, the interior of a bone 10 is hollowed out in preparation for insertion of the implant 12. The implant has a contoured surface design 14 defining plural indentations 16 to permit ingrowth of bone into the indentations. The indentations are preferably transverse to the longitudinal axis 18 of the implant. The contoured portion to be inserted in the orifice may be coated with osteogenic protein as described above. Osteogenic protein combined with a matrix material 20 is packed into the orifice with the prosthetic implant, thereby surrounding it. Stimulated by the osteogenic protein, new bone grows into the indentations 16 and becomes integrated with the surface of the implant 12 and with preexisting bone 10 as described above. Thus, the prosthesis is both mechanically and biologically fixed in place, and axial movement of the implant relative to the bone requires shearing of bone tissue. Matrix material 20 can be any of the materials described above for coating the prosthesis for enhancing bone growth and fixation, e.g., collagen, hydroxyapatite, homopolymers or copolymers of glycolic acid lactic acid, and butyric acid and derivatives thereof, tricalcium phosphate or other calcium phosphates, metal oxides and demineralized, guanidine extracted bone. Matrix materials for use with osteogenic proteins which can be used in the present embodiment are those described, for example, in U.S. Patent 5,011,691 and in copending U.S. patent application Serial No. 07/841,646 by Oppermann et al., filed February 21, 1992, the teachings of which are hereby incorporated by reference.

The prosthesis illustrated in the Figure is particularly useful for dental and other implants where at least part of the prosthesis is to be embedded into bone tissue. Packing the orifice, e.g., tooth socket, with an "osteogenic

- 23 -

device," e.g., osteogenic protein in combination with a matrix material, provides a solid material in which to embed the prosthesis without requiring that the device be threaded into existing bone. Moreover, the osteogenic protein stimulates endochondral bone formation within the socket and into and around the implant, thereby obviating the previously required step of first allowing bone ingrowth into the socket in order to provide a suitable surface into which to implant the prosthesis. Accordingly, using the method and devices of the invention, strong fixation of an implanted prosthesis may be achieved in a fraction of the time previously required, significantly shortening the time interval between tooth extraction and prosthetic restoration. In addition, this treatment may expand the use of implant therapy and enhance success rates by eliminating a surgical procedure, reducing the amount of bone lost following tooth extraction, permitting the insertion of longer implants and minimizing prosthetic compromises necessitated by alveolar ridge resorption.

The invention will be further illustrated by the following Exemplification which is not intended to be limiting in any way.

EXEMPLIFICATION

Example 1

Metal Implant Fixation

Cylindrical implants 18mm in length and 5.95 ± 0.05 mm in diameter were fabricated from spherical Co-Cr-Mo particles resulting in a pore size of 250-300 μ m and a volume porosity of 38-40%. A highly crystalline, high density and low porosity hydroxylapatite (HA) coating was applied by plasma spray process to one-half of the length of each of the implants. The coating thickness was 25 μ m and did not alter the porous coating morphology.

- 24 -

In the initial study, three implants were treated with a partially purified bovine OP (bOP) preparation. The bOP was naturally sourced OP extracted from cortical bone and partially purified through the Sephacryl-300 HR step in the purification protocol as described in Sampath et al. (1990), J. Biol. Chem., 265: 13198-13205. 200 μ l aliquots of 4 M guanidine-HCl, 50 mM Tris-HCl, pH 7.0, containing approximately 80 μ g bOP were added to each implant in an eppendorf tube. After overnight incubation at 4°C the protein was precipitated and the implant washed with 80% ethanol. The implants were subsequently freeze dried. Two implants without bOP served as the controls.

The implants were evaluated in one skeletally mature adult mongrel dog (3-5 years old, 20-25Kg weight) using the femoral transcortical model. Standard surgical techniques were used such that the animal received the five implants in one femur. At three weeks the dog was sacrificed and the femur removed.

The harvested femur was sectioned transverse to the long axis such that each implant was isolated. Each implant was sectioned in half to yield one HA-coated and one uncoated push-out sample. Interface attachment strength was determined using a specifically designed test fixture. The implants were pushed to failure with a MTS test machine at a displacement rate of 1.27 mm/minute. After testing, all samples were prepared for standard undecalcified histologic and microradiographic analyses. The sections (4 sections from each implant) were qualitatively examined for the type and quality of tissue ingrowth, and quantitatively evaluated for % bone ingrowth with a computerized image analysis system. The mechanical and quantitative histological data is shown in Table II.

- 25 -

TABLE II
METAL IMPLANTS - bOP

3 WEEKS

	HA-Coated	Uncoated
Interface Shear Strength, MPa		
Control	9.70 (n=2)	3.40 (n=2)
Protein (bOP)	10.75 (n=3)	4.08 (n=3)
Percent Bone Ingrowth		
Control	42.56 (n=4)	37.82 (n=4)
Protein (bOP)	51.66 (n=4)	46.38 (n=4)

Both the mechanical and histological data suggested that bOP enhanced osseointegration of the implants. Both the HA-coated and uncoated implants showed an increase of shear strength and bone ingrowth compared with untreated controls. Moreover, the HA-coated implants appeared to show significant enhancement compared to the uncoated implant. The histological sections directly showed a greater number of cells between the metal pores.

The positive results of the initial implant study prompted a more detailed study. Twenty-seven implants were treated with a recombinant human OP1 protein. The OP1 protein was produced by transformed CHO cells. Details for the recombinant production of OP1 are disclosed in USSN 841,646, incorporated hereinabove by reference. The protein was purified to contain as the major species the protein designated OP1-18Ser (Seq. ID No. 1, residues 293-431), and about 30% truncated forms of OP1 (e.g., OP1-16Ser, OP1-16Leu, OP1-16Met, OP1-16Ala and OP1-16Val). The protein was greater than 90% pure. The implants were immersed for 30 minutes in

- 26 -

200 μ l 50% ethanol/0.01% TFA containing 5 μ g recombinant protein and the solution frozen in an ethanol/dry ice bath while the formulation tube was rolled. The tubes were subsequently freeze dried. Nineteen implants were also prepared by treatment with ethanol/TFA without the OP1 protein by the same procedure.

In test implants, it was found that OP1 could be extracted from treated implants with 8M urea, 1% Tween 80, 50mM Tris, pH 8.0 and analyzed by HPLC. By this method, it was shown that all of the OP1 in the formulation tubes bound to the implant under the conditions employed. Furthermore, since the test implants were half coated with HA, additional implants were obtained to independently evaluate the binding of OP1 to each of these surfaces. Initial binding studies showed that the OP1 binds more readily to the HA than to the uncoated metal.

The implants for the second study were evaluated in skeletally mature adult mongrel dogs using the femoral transcortical model. Standard aseptic surgical techniques were used such that each animal received five implants bilaterally. Implantation periods of three weeks were used. The mechanical and quantitative histological data are shown in Table III. Three HA-coated and uncoated configurations were evaluated: controls (no treatment), precoat samples (formulated without OP1) and the OP1 samples.

- 27 -

TABLE III
METAL IMPLANTS - OP-1

	<u>INTERFACE SHEAR ATTACHMENT STRENGTH, MPA</u>		<u>PERCENT BONE INGROWTH</u>	
	3 Weeks:		3 Weeks:	
	<u>HA-coated</u>	<u>Uncoated</u>	<u>HA-coated</u>	<u>Uncoated</u>
Control	7.59+2.99 (\bar{n} =10)	6.47+1.23 (\bar{n} =10)	44.98+12.57 (\bar{n} =24)	41.66+11.91 (\bar{n} =24)
Precoat	7.85+3.43 (\bar{n} =9)	6.49+2.20 (\bar{n} =9)	40.73+16.88 (\bar{n} =24)	39.14+16.18 (\bar{n} =24)
Protein (hOP-1)	8.69+3.17 (\bar{n} =17)	6.34+3.04 (\bar{n} =17)	48.68+16.61 (\bar{n} =24)	47.89+11.91 (\bar{n} =24)

Mechanical testing results demonstrated enhanced attachment strength for the HA-coated samples as compared to the uncoated samples. At three weeks the greatest fixation was observed with the HA-coated implant with protein.

Histologic analysis demonstrated greater bone ingrowth for all HA-coated versus uncoated samples although the differences were not significant. The percent bone ingrowth was greatest for the HA-coated and uncoated implants with the protein present. Linear regression analysis demonstrated that attachment strength was predicted by amount of bone growth into the porous structure, presence of HA coating, and presence of protein.

Example 2

Titanium frequently is used to fabricate metal prostheses. The surface of these prostheses comprise a layer of titanium oxide. Therefore, titanium oxide itself was evaluated for its ability to serve as a carrier for OP-1 and in general for its biocompatibility with the bone formation process. The in vivo biological activity of implants containing a combination of titanium oxide and OP-1 (Sequence ID No. 1, residues 293-431)

- 28 -

was examined in rat subcutaneous and intramuscular assays. Implants contained 0, 6.25, 12.5, 25 or 50 μg of OP-1 formulated onto 30 mg of titanium oxide.

Implants were formulated by a modification of the ethanol/TFA freeze-drying method. Titanium oxide pellets were milled and sieved to a particle size of 250-420 microns. 30 mg of these particles were mixed with 50 μl aliquots of 45% ethanol, 0.09% trifluoroacetic acid containing no OP-1 or various concentrations of OP-1. After 3 hours at 4 °C, the samples were frozen, freeze-dried and implanted into rats.

After 12 days in vivo the implants were removed and evaluated for bone formation by alkaline phosphatase specific activity, calcium content and histological evidence. The results showed that OP-1 induced the formation of bone at each concentration of OP-1 at both the subcutaneous and intramuscular implant sites. No bone formed without OP-1 added to the titanium oxide. The amount of bone as quantitated by calcium content of the implants was similar to that observed using bone collagen carriers. Therefore titanium is a useful carrier for osteogenic proteins and is biocompatible with the bone formation process.

Example 3

The efficacy of the method of this invention on standard dental prosthesis may be assessed using the following model and protocol. Maxillary and mandibular incisor and mandibular canine teeth are extracted from several (e.g., 3) male cynomolgus (*Macaca fascicularis*) monkeys (4-6 kilograms) under ketamine anesthesia and local infiltration of lidocaine. Hemostasis is achieved with pressure.

The resultant toothless sockets are filled either with (a) collagen matrix (CM), (b) with collagen matrix containing osteogenic protein, such as the recombinantly produced OP1 protein used in Example 1, above (e.g., an osteogenic device) or c) are left untreated. Titanium, self-tapping, oral,

- 29 -

endosseous implants (Nobelpharma, Chicago, Ill.) are inserted into all of the sockets by minimally engaging the self-tapping tip. The mucoperiosteal flap is released from the underlying tissue and used to obtain primary wound closure using standard surgical procedures known in the medical art.

The animals are sacrificed after three weeks by lethal injection of pentobarbital and perfusion with paraformaldehyde-glutaraldehyde. The jaws then are dissected and the blocks containing the appropriate sockets are resected, further fixed in neutral buffered formalin, decalcified in formic acid and sodium citrate, embedded in plastic and stained with basic Fuchsin and toluidine blue. Sections then are analyzed by light microscopy. Preferably, computer assisted histomorphometric analysis is used to evaluate the new tissue, e.g., using Image 1.27 and Quick Capture^R (Data Translation, Inc. Marlboro, MA 07152).

It is anticipated that sockets which contain the osteogenic device will induce the formation of new bone in close apposition to the threaded surface of the titanium implants within 3 weeks. By contrast, sockets treated only with collagen matrix or sockets receiving neither collagen matrix nor the osteogenic device should show no evidence of new bone formation in close apposition to the implant surface.

- 30 -

Equivalents

One skilled in the art will be able to ascertain, using no more than routine experimentation, many equivalents to the subject matter described herein. Such equivalents are intended to be encompassed by the following claims.

- 31 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Creative BioMolecules, Inc.
(B) STREET: 35 South Street
(C) CITY: Hopkinton
(D) STATE: Massachusetts
(E) COUNTRY: United States
(F) POSTAL CODE (ZIP): 01748
(G) TELEPHONE: 1-508-435-9001
(H) TELEFAX: 1-508-435-0454
(I) TELEX:

(A) NAME: Stryker Biotech
(B) STREET: One Apple Hill
(C) CITY: Natick
(D) STATE: Massachusetts
(E) COUNTRY: United States
(F) POSTAL CODE (ZIP): 01760
(G) TELEPHONE: 1-508-653-2280
(H) TELEFAX: 1-508-653-2770
(I) TELEX:

(ii) TITLE OF INVENTION: PROSTHETIC DEVICES HAVING ENHANCED
OSTEOGENIC PROPERTIES

(iii) NUMBER OF SEQUENCES: 22

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Creative BioMolecules, Inc.
(B) STREET: 35 South Street
(C) CITY: Hopkinton
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 01748

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

- 32 -

- (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: PITCHER ESQ, EDMUND R
(B) REGISTRATION NUMBER: 27,829
(C) REFERENCE/DOCKET NUMBER: STK-057

- (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617/248-7000

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1822 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS
(F) TISSUE TYPE: HIPPOCAMPUS
- (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 49..1341
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
/product= "OP1"
/evidence= EXPERIMENTAL
/standard_name= "OP1"

- 33 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGTGCGGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCGCG ATG CAC GTG	57
Met His Val	
1	
CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA	105
Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala	
5 10 15	
CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC	153
Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn	
20 25 30 35	
GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG	201
Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg	
40 45 50	
CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC	249
Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg	
55 60 65	
CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG	297
Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met	
70 75 80	
CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC	345
Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly	
85 90 95	
GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC	393
Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly	
100 105 110 115	

- 34 -

CCC	CCT	CTG	GCC	AGC	CTG	CAA	GAT	AGC	CAT	TTC	CTC	ACC	GAC	GCC	GAC	441
Pro	Pro	Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	Asp	Ala	Asp	
				120					125					130		
ATG	GTC	ATG	AGC	TTC	GTC	AAC	CTC	GTG	GAA	CAT	GAC	AAG	GAA	TTC	TTC	489
Met	Val	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	Glu	Phe	Phe	
			135					140					145			
CAC	CCA	CGC	TAC	CAC	CAT	CGA	GAG	TTC	CGG	TTT	GAT	CTT	TCC	AAG	ATC	537
His	Pro	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	
			150				155					160				
CCA	GAA	GGG	GAA	GCT	GTC	ACG	GCA	GCC	GAA	TTC	CGG	ATC	TAC	AAG	GAC	585
Pro	Glu	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	
	165					170					175					
TAC	ATC	CGG	GAA	CGC	TTC	GAC	AAT	GAG	ACG	TTC	CGG	ATC	AGC	GTT	TAT	633
Tyr	Ile	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Arg	Ile	Ser	Val	Tyr	
180					185					190					195	
CAG	GTG	CTC	CAG	GAG	CAC	TTG	GGC	AGG	GAA	TCG	GAT	CTC	TTC	CTG	CTC	681
Gln	Val	Leu	Gln	Glu	His	Leu	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	
				200				205						210		
GAC	AGC	CGT	ACC	CTC	TGG	GCC	TCG	GAG	GAG	GGC	TGG	CTG	GTG	TTT	GAC	729
Asp	Ser	Arg	Thr	Leu	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe	Asp	
			215					220					225			
ATC	ACA	GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAT	CCG	CGG	CAC	AAC	CTG	777
Ile	Thr	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	
		230					235					240				
GGC	CTG	CAG	CTC	TCG	GTG	GAG	ACG	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	825
Gly	Leu	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	
	245					250					255					
AAG	TTG	GCG	GGC	CTG	ATT	GGG	CGG	CAC	GGG	CCC	CAG	AAC	AAG	CAG	CCC	873
Lys	Leu	Ala	Gly	Leu	Ile	Gly	Arg	His	Gly	Pro	Gln	Asn	Lys	Gln	Pro	
260					265					270					275	
TTC	ATG	GTG	GCT	TTC	TTC	AAG	GCC	ACG	GAG	GTC	CAC	TTC	CGC	AGC	ATC	921
Phe	Met	Val	Ala	Phe	Phe	Lys	Ala	Thr	Glu	Val	His	Phe	Arg	Ser	Ile	
				280					285					290		
CGG	TCC	ACG	GGG	AGC	AAA	CAG	CGC	AGC	CAG	AAC	CGC	TCC	AAG	ACG	CCC	969
Arg	Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln	Asn	Arg	Ser	Lys	Thr	Pro	
			295					300					305			
AAG	AAC	CAG	GAA	GCC	CTG	CGG	ATG	GCC	AAC	GTG	GCA	GAG	AAC	AGC	AGC	1017
Lys	Asn	Gln	Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala	Glu	Asn	Ser	Ser	
		310					315					320				

- 35 -

AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe 325 330 335	1065
CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala 340 345 350 355	1113
GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met 360 365 370	1161
AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn 375 380 385	1209
CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala 390 395 400	1257
ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys 405 410 415	1305
TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC Tyr Arg Asn Met Val Arg Ala Cys Gly Cys His 420 425 430	1351
GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG	1411
GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG	1471
TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC	1531
ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC	1591
GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT	1651
CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG	1711
GGCGTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC	1771
CTGTAATAAA TGTCACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A	1822

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 431 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- 36 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
1 5 10 15
Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
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Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
35 40 45
Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
50 55 60
Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
65 70 75 80
Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
85 90 95
Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
100 105 110
Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
115 120 125
Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
130 135 140
Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
145 150 155 160
Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
165 170 175
Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
180 185 190
Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
195 200 205
Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
210 215 220
Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
225 230 235 240
His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
245 250 255
Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
260 265 270

- 37 -

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
 275 280 285
 Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
 290 295 300
 Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
 305 310 315 320
 Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
 325 330 335
 Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
 340 345 350
 Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
 355 360 365
 Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
 370 375 380
 Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
 385 390 395 400
 Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
 405 410 415
 Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
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(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 96 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..96
- (D) OTHER INFORMATION: /note= "COP-5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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 1 5 10 15
 Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Glu Cys Pro Phe Pro
 20 25 30

- 38 -

Leu Ala Asp His Phe Asn Ser Thr Asn His Ala Val Val Gln Thr Leu
35 40 45

Val	Asn	Ser	Val	Asn	Ser	Lys	Ile	Pro	Lys	Ala	Cys	Cys	Val	Pro	Thr
50						55					60				

Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu Asn Glu Lys Val
65 70 75 80

Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu Gly Cys Gly Cys Arg
85 90 95

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 96 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) **FEATURE:**

(A) NAME/KEY: Protein

(B) LOCATION: 1..96

(D) OTHER INFORMATION: /note= "COP-7"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly Glu Cys Pro Phe Pro
20 25 30

Leu Ala Asp His Leu Asn Ser Thr Asn His Ala Val Val Gln Thr Leu
35 40 45

Val	Asn	Ser	Val	Asn	Ser	Lys	Ile	Pro	Lys	Ala	Cys	Cys	Val	Pro	Thr
50						55					60				

Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu Asn Glu Lys Val
65 70 75 80

Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu Gly Cys Gly Cys Arg
85 90 95

- 39 -

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: DROSOPHILA MELANOGASTER

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..101
- (D) OTHER INFORMATION: /label= DPP-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asp
1          5          10          15
Asp Trp Ile Val Ala Pro Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly
          20          25          30
Lys Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser Thr Asn His Ala
          35          40          45
Val Val Gln Thr Leu Val Asn Asn Asn Asn Pro Gly Lys Val Pro Lys
          50          55          60
Ala Cys Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met Leu Tyr Leu
65          70          75          80
Asn Asp Gln Ser Thr Val Val Leu Lys Asn Tyr Gln Glu Met Thr Val
          85          90          95
Val Gly Cys Gly Cys Arg
          100

```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- 40 -

(vi) ORIGINAL SOURCE:

(A) ORGANISM: XENOPUS

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= VG1-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys Asp Val Gly Trp Gln
1           5           10           15
Asn Trp Val Ile Ala Pro Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly
          20           25           30
Glu Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly Ser Asn His Ala
          35           40           45
Ile Leu Gln Thr Leu Val His Ser Ile Glu Pro Glu Asp Ile Pro Leu
          50           55           60
Pro Cys Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met Leu Phe Tyr
65           70           75           80
Asp Asn Asn Asp Asn Val Val Leu Arg His Tyr Glu Asn Met Ala Val
          85           90           95
Asp Glu Cys Gly Cys Arg
          100

```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: MURIDAE

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= VGR-1-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

(2) INFORMATION FOR SEQ ID NO:8:

(A) LENGTH: 1873 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: MURIDAE
(F) TISSUE TYPE: EMBRYO

(A) NAME/KEY: CDS
(B) LOCATION: 104..1393
(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
/product= "MOP1"
/note= "MOP1 (CDNA)"

- 42 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG	60
CGGCGCGGGC CCGGTGCCCC GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC	115
Met His Val Arg	
1	
TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT	163
Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro	
5 10 15 20	
CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG	211
Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu	
25 30 35	
GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG	259
Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg	
40 45 50	
GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG	307
Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro	
55 60 65	
CGC CCG CAC CTC CAG GGA AAG CAT AAT TCG GCG CCC ATG TTC ATG TTG	355
Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu	
70 75 80	
GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG AGC GGG CCG GAC GGA CAG	403
Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly Pro Asp Gly Gln	
85 90 95 100	
GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT	451
Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro	
105 110 115	
TTA GCC AGC CTG CAG GAC AGC CAT TTC CTC ACT GAC GCC GAC ATG GTC	499
Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp Met Val	
120 125 130	
ATG AGC TTC GTC AAC CTA GTG GAA CAT GAC AAA GAA TTC TTC CAC CCT	547
Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro	
135 140 145	
CGA TAC CAC CAT CGG GAG TTC CGG TTT GAT CTT TCC AAG ATC CCC GAG	595
Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu	
150 155 160	
GGC GAA CGG GTG ACC GCA GCC GAA TTC AGG ATC TAT AAG GAC TAC ATC	643
Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile	
165 170 175 180	

- 43 -

CGG GAG CGA TTT GAC AAC GAG ACC TTC CAG ATC ACA GTC TAT CAG GTG	691
Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr Val Tyr Gln Val	
185 190 195	
CTC CAG GAG CAC TCA GGC AGG GAG TCG GAC CTC TTC TTG CTG GAC AGC	739
Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe Leu Leu Asp Ser	
200 205 210	
CGC ACC ATC TGG GCT TCT GAG GAG GGC TGG TTG GTG TTT GAT ATC ACA	787
Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp Ile Thr	
215 220 225	
GCC ACC AGC AAC CAC TGG GTG GTC AAC CCT CGG CAC AAC CTG GGC TTA	835
Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu Gly Leu	
230 235 240	
CAG CTC TCT GTG GAG ACC CTG GAT GGG CAG AGC ATC AAC CCC AAG TTG	883
Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro Lys Leu	
245 250 255 260	
GCA GGC CTG ATT GGA CGG CAT GGA CCC CAG AAC AAG CAA CCC TTC ATG	931
Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met	
265 270 275	
GTG GCC TTC TTC AAG GCC ACG GAA GTC CAT CTC CGT AGT ATC CGG TCC	979
Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg Ser Ile Arg Ser	
280 285 290	
ACG GGG GGC AAG CAG CGC AGC CAG AAT CGC TCC AAG ACG CCA AAG AAC	1027
Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn	
295 300 305	
CAA GAG GCC CTG AGG ATG GCC AGT GTG GCA GAA AAC AGC AGC AGT GAC	1075
Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp	
310 315 320	
CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC TTC CGA GAC	1123
Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp	
325 330 335 340	
CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC	1171
Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr	
345 350 355	
TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC	1219
Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala	
360 365 370	
ACC AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC	1267
Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp	
375 380 385	

- 44 -

ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT	1315
Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser	
390 395 400	
GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC GAC CTG AAG AAG TAC AGA	1363
Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Asp Leu Lys Lys Tyr Arg	
405 410 415 420	
AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCTTCC TGAGACCCTG	1413
Asn Met Val Val Arg Ala Cys Gly Cys His	
425 430	
ACCTTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG	1473
CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCTGAGCC TTCCCTCACC TCCCAACCGG	1533
AAGCATGTAA GGGTTCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCCTTCT	1593
GGCAGGTGAC GGACAAGATC CTACCAGCTA CCACAGCAAA CGCCTAAGAG CAGGAAAAAT	1653
GTCTGCCAGG AAAGTGTCCA GTGTCCACAT GGCCCCTGGC GCTCTGAGTC TTTGAGGACT	1713
AATCGCAAGC CTCGTTGAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GGCGCTGGCG	1773
TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAACCCAT	1833
GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTC	1873

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 430 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala	
1 5 10 15	
Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser	
20 25 30	
Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser	
35 40 45	
Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu	
50 55 60	

- 45 -

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
 65 70 75 80
 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly
 85 90 95
 Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr
 100 105 110
 Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp
 115 120 125
 Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu
 130 135 140
 Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser
 145 150 155 160
 Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr
 165 170 175
 Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr
 180 185 190
 Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe
 195 200 205
 Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val
 210 215 220
 Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His
 225 230 235 240
 Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile
 245 250 255
 Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys
 260 265 270
 Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg
 275 280 285
 Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys
 290 295 300
 Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn
 305 310 315 320
 Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val
 325 330 335
 Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly
 340 345 350

- 46 -

Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser
 355 360 365
 Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe
 370 375 380
 Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu
 385 390 395 400
 Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Asp Leu
 405 410 415
 Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1723 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens
 - (F) TISSUE TYPE: HIPPOCAMPUS
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 490..1696
 - (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "hOP2-PP"
 /note= "hOP2 (cDNA)"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGGTTG GAGCAGGAGG TGGCACGGCA	60
GGGCTGGAGG GCTCCCTATG AGTGGCGGAG ACGGCCAGG AGGCGCTGGA GCAACAGCTC	120
CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTCGCCCATC GCCCCTGCGC TGCTCGGACC	180
GCGGCCACAG CCGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCAGT	240
CCGCAGAGTA GCGCCGGCCT CGAGGCGGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG	300
GACAGGTGTC GCGCGGCGGG GCTCCAGGGA CCGCGCCTGA GGCCGGCTGC CCGCCCGTCC	360
CGCCCCGCCC CGCCGCCCCG CGCCGCCGA GCCCAGCCTC CTTGCCGTCG GGGCGTCCCC	420

- 47 -

AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCCGCTGA GCGCCCCAGC TGAGCGCCCC	480
CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG	528
Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu	
1 5 10	
GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG CGA CCC CCG CCC	576
Ala Leu Cys Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro	
15 20 25	
GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG	624
Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln	
30 35 40 45	
CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC	672
Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg	
50 55 60	
GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG CTC TTC ATG	720
Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met	
65 70 75	
CTG GAC CTG TAC CAC GCC ATG GCC GGC GAC GAC GAC GAG GAC GGC GCG	768
Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala	
80 85 90	
CCC GCG GAG CGG CGC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC GTT	816
Pro Ala Glu Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val	
95 100 105	
AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT TGG	864
Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp	
110 115 120 125	
AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG GTC	912
Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val	
130 135 140	
ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC CTG CTC	960
Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu	
145 150 155	
AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC	1008
Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser	
160 165 170	
AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA GCT	1056
Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala	
175 180 185	

- 48 -

GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC TGC Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys 190 195 200 205	1104
TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG GAG Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu 210 215 220	1152
ACT GAG GAC GGG CAC AGC GTG GAT CCT GGC CTG GCC GGC CTG CTG GGT Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly 225 230 235	1200
CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC GTG GTC ACT TTC TTC AGG Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg 240 245 250	1248
GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG GCA GTG AGG CCA CTG AGG Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg 255 260 265	1296
AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA CTC Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu 270 275 280 285	1344
CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC TGC Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys 290 295 300	1392
CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG GAC Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp 305 310 315	1440
TGG GTC ATC GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG GAG Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu 320 325 330	1488
TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC ATC Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile 335 340 345	1536
CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala 350 355 360 365	1584
TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp 370 375 380	1632
AGC AGC AAC AAC GTC ATC CTG CGC AAA GCC CGC AAC ATG GTG GTC AAG Ser Ser Asn Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val Lys 385 390 395	1680

- 49 -

GCC TGC GGC TGC CAC T GAGTCAGCCC GCCCAGCCCT ACTGCAG
 Ala Cys Gly Cys His
 400

1723

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 402 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
 1 5 10 15
 Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro
 20 25 30
 Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln Arg Glu Ile
 35 40 45
 Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro
 50 55 60
 Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu
 65 70 75 80
 Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu
 85 90 95
 Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val
 100 105 110
 Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe
 115 120 125
 Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala
 130 135 140
 Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr
 145 150 155 160
 Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu
 165 170 175
 Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu
 180 185 190

- 50 -

Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu
 195 200 205
 Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp
 210 215 220
 Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala
 225 230 235 240
 Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro
 245 250 255
 Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln
 260 265 270
 Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile
 275 280 285
 Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His
 290 295 300
 Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile
 305 310 315 320
 Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe
 325 330 335
 Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser
 340 345 350
 Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala
 355 360 365
 Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn
 370 375 380
 Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val Lys Ala Cys Gly
 385 390 395 400
 Cys His

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1926 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: MURIDAE
 - (F) TISSUE TYPE: EMBRYO

- 51 -

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 93..1289

(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"

/product= "mOP2-PP"

/note= "mOP2 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC CCGACCAGCT	60
ACCACTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT CCC GGG CCA	113
Met Ala Met Arg Pro Gly Pro	
1 5	
CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC GGC CAC GGT	161
Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly Gly His Gly	
10 15 20	
CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA GCG CGC GAG	209
Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu	
25 30 35	
CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG CTA CCG GGA	257
Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly	
40 45 50 55	
CGG CCC CGA CCC CGT GCA CAA CCC GCC GCT GCC CGG CAG CCA GCG TCC	305
Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Ala Arg Gln Pro Ala Ser	
60 65 70	
GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC GAT GAC GAC	353
Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr Asp Asp Asp	
75 80 85	
GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC CTG GTC ATG	401
Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp Leu Val Met	
90 95 100	
AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC TAC CAG GAG	449
Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly Tyr Gln Glu	
105 110 115	
CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC CCT GCT GGG	497
Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile Pro Ala Gly	
120 125 130 135	
GAG GCT GTC ACA GCT GCT GAG TTC CGG ATC TAC AAA GAA CCC AGC ACC	545
Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu Pro Ser Thr	
140 145 150	

- 52 -

CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA GTG GTC CAA	593
His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu Val Val Gln	
155 160 165	
GAG CAC TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG	641
Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr	
170 175 180	
CTC CGA TCT GGG GAC GAG GGC TGG CTG GTG CTG GAC ATC ACA GCA GCC	689
Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile Thr Ala Ala	
185 190 195	
AGT GAC CGA TGG CTG CTG AAC CAT CAC AAG GAC CTG GGA CTC CGC CTC	737
Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly Leu Arg Leu	
200 205 210 215	
TAT GTG GAA ACC GCG GAT GGG CAC AGC ATG GAT CCT GGC CTG GCT GGT	785
Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly Leu Ala Gly	
220 225 230	
CTG CTT GGA CGA CAA GCA CCA CGC TCC AGA CAG CCT TTC ATG GTA ACC	833
Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe Met Val Thr	
235 240 245	
TTC TTC AGG GCC AGC CAG AGT CCT GTG CGG GCC CCT CGG GCA GCG AGA	881
Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg Ala Ala Arg	
250 255 260	
CCA CTG AAG AGG AGG CAG CCA AAG AAA ACG AAC GAG CTT CCG CAC CCC	929
Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu Pro His Pro	
265 270 275	
AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC CGC GGC AGA	977
Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser Arg Gly Arg	
280 285 290 295	
GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGC TTC CGT GAC CTT GGC	1025
Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly	
300 305 310	
TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC TAT TAC TGT	1073
Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys	
315 320 325	
GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC GCC ACC AAC	1121
Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn	
330 335 340	
CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA GAT GTT GTC	1169
His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asp Val Val	
345 350 355	

- 53 -

CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC TCT GTG CTG	1217
Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu	
360 365 370 375	
TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC CGT AAC ATG	1265
Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met	
380 385 390	
GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCG CCCAGCATCC TGCTTCTACT	1319
Val Val Lys Ala Cys Gly Cys His	
395	
ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT TATCATAGCT	1379
CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCTGCTA AAATTCTGGT	1439
CTTTCCCACT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC CTCTCCATCC	1499
TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA ACTGAGAGGT	1559
CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC CTCAGCCCAC	1619
AATGGCAAAT TCTGGATGGT CTAAGAAGGC CCTGGAATTC TAAACTAGAT GATCTGGGCT	1679
CTCTGCACCA TTCATTGTGG CAGTTGGGAC ATTTTITAGGT ATAACAGACA CATACTTA	1739
GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA AGAATCAGAG	1799
CCAGGTATAG CGGTGCATGT CATTAAATCCC AGCGCTAAAG AGACAGAGAC AGGAGAATCT	1859
CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA AAAAAAAAC	1919
GGAATTC	1926

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 399 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met	Ala	Met	Arg	Pro	Gly	Pro	Leu	Trp	Leu	Leu	Gly	Leu	Ala	Leu	Cys
1				5					10					15	
Ala	Leu	Gly	Gly	Gly	His	Gly	Pro	Arg	Pro	Pro	His	Thr	Cys	Pro	Gln
		20						25					30		

- 54 -

Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu
 35 40 45
 Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala
 50 55 60
 Ala Ala Arg Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr
 65 70 75 80
 His Ala Met Thr Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu
 85 90 95
 Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp
 100 105 110
 Arg Thr Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp
 115 120 125
 Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg
 130 135 140
 Ile Tyr Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile
 145 150 155 160
 Ser Met Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu
 165 170 175
 Phe Phe Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu
 180 185 190
 Val Leu Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His
 195 200 205
 Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser
 210 215 220
 Met Asp Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser
 225 230 235 240
 Arg Gln Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val
 245 250 255
 Arg Ala Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys
 260 265 270
 Thr Asn Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp
 275 280 285
 Gly His Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr
 290 295 300
 Val Ser Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln
 305 310 315 320

- 55 -

Gly	Tyr	Ser	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asp
				325					330					335	
Ser	Cys	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	Leu	Gln	Ser	Leu	Val	His
			340					345					350		
Leu	Met	Lys	Pro	Asp	Val	Val	Pro	Lys	Ala	Cys	Cys	Ala	Pro	Thr	Lys
		355					360					365			
Leu	Ser	Ala	Thr	Ser	Val	Leu	Tyr	Tyr	Asp	Ser	Ser	Asn	Asn	Val	Ile
	370					375					380				
Leu	Arg	Lys	His	Arg	Asn	Met	Val	Val	Lys	Ala	Cys	Gly	Cys	His	
385					390					395					

(2) INFORMATION FOR SEQ ID NO:14:

- ```

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1260 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 9..1196
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "BMP2A"
 /note= "BMP2A (CDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```

GGTCGACC ATG GTG GCC GGG ACC CGC TGT CTT CTA GCG TTG CTG CTT CCC 50  
Met Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro  
1 5 10

CAG GTC CTC CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC CGC 98  
Gln Val Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg  
15 20 25 30

- 56 -

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| AGG | AAG | TTC | GCG | GCG | GCG | TCG | TCG | GGC | CGC | CCC | TCA | TCC | CAG | CCC | TCT | 146 |
| Arg | Lys | Phe | Ala | Ala | Ala | Ser | Ser | Gly | Arg | Pro | Ser | Ser | Gln | Pro | Ser |     |
|     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
| GAC | GAG | GTC | CTG | AGC | GAG | TTC | GAG | TTG | CGG | CTG | CTC | AGC | ATG | TTC | GGC | 194 |
| Asp | Glu | Val | Leu | Ser | Glu | Phe | Glu | Leu | Arg | Leu | Leu | Ser | Met | Phe | Gly |     |
|     |     |     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |
| CTG | AAA | CAG | AGA | CCC | ACC | CCC | AGC | AGG | GAC | GCC | GTG | GTG | CCC | CCC | TAC | 242 |
| Leu | Lys | Gln | Arg | Pro | Thr | Pro | Ser | Arg | Asp | Ala | Val | Val | Pro | Pro | Tyr |     |
|     |     | 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     |
| ATG | CTA | GAC | CTG | TAT | CGC | AGG | CAC | TCG | GGT | CAG | CCG | GGC | TCA | CCC | GCC | 290 |
| Met | Leu | Asp | Leu | Tyr | Arg | Arg | His | Ser | Gly | Gln | Pro | Gly | Ser | Pro | Ala |     |
|     | 80  |     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     |     |
| CCA | GAC | CAC | CGG | TTG | GAG | AGG | GCA | GCC | AGC | CGA | GCC | AAC | ACT | GTG | CGC | 338 |
| Pro | Asp | His | Arg | Leu | Glu | Arg | Ala | Ala | Ser | Arg | Ala | Asn | Thr | Val | Arg |     |
|     | 95  |     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |
| AGC | TTC | CAC | CAT | GAA | GAA | TCT | TTG | GAA | GAA | CTA | CCA | GAA | ACG | AGT | GGG | 386 |
| Ser | Phe | His | His | Glu | Glu | Ser | Leu | Glu | Glu | Leu | Pro | Glu | Thr | Ser | Gly |     |
|     |     |     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |
| AAA | ACA | ACC | CGG | AGA | TTC | TTC | TTT | AAT | TTA | AGT | TCT | ATC | CCC | ACG | GAG | 434 |
| Lys | Thr | Thr | Arg | Arg | Phe | Phe | Phe | Asn | Leu | Ser | Ser | Ile | Pro | Thr | Glu |     |
|     |     |     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |
| GAG | TTT | ATC | ACC | TCA | GCA | GAG | CTT | CAG | GTT | TTC | CGA | GAA | CAG | ATG | CAA | 482 |
| Glu | Phe | Ile | Thr | Ser | Ala | Glu | Leu | Gln | Val | Phe | Arg | Glu | Gln | Met | Gln |     |
|     |     | 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     |
| GAT | GCT | TTA | GGA | AAC | AAT | AGC | AGT | TTC | CAT | CAC | CGA | ATT | AAT | ATT | TAT | 530 |
| Asp | Ala | Leu | Gly | Asn | Asn | Ser | Ser | Phe | His | His | Arg | Ile | Asn | Ile | Tyr |     |
|     | 160 |     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     |     |
| GAA | ATC | ATA | AAA | CCT | GCA | ACA | GCC | AAC | TCG | AAA | TTC | CCC | GTG | ACC | AGT | 578 |
| Glu | Ile | Ile | Lys | Pro | Ala | Thr | Ala | Asn | Ser | Lys | Phe | Pro | Val | Thr | Ser |     |
|     | 175 |     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |
| CTT | TTG | GAC | ACC | AGG | TTG | GTG | AAT | CAG | AAT | GCA | AGC | AGG | TGG | GAA | AGT | 626 |
| Leu | Leu | Asp | Thr | Arg | Leu | Val | Asn | Gln | Asn | Ala | Ser | Arg | Trp | Glu | Ser |     |
|     |     |     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |
| TTT | GAT | GTC | ACC | CCC | GCT | GTG | ATG | CGG | TGG | ACT | GCA | CAG | GGA | CAC | GCC | 674 |
| Phe | Asp | Val | Thr | Pro | Ala | Val | Met | Arg | Trp | Thr | Ala | Gln | Gly | His | Ala |     |
|     |     |     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |
| AAC | CAT | GGA | TTC | GTG | GTG | GAA | GTG | GCC | CAC | TTG | GAG | GAG | AAA | CAA | GGT | 722 |
| Asn | His | Gly | Phe | Val | Val | Glu | Val | Ala | His | Leu | Glu | Glu | Lys | Gln | Gly |     |
|     |     | 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     |

- 57 -

|                                                                                                                                                       |      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG CAC CAA GAT GAA<br>Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu His Gln Asp Glu<br>240 245 250     | 770  |
| CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC CAT GAT<br>His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly His Asp<br>255 260 265 270 | 818  |
| GGA AAA GGG CAT CCT CTC CAC AAA AGA GAA AAA CGT CAA GCC AAA CAC<br>Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His<br>275 280 285     | 866  |
| AAA CAG CGG AAA CGC CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC<br>Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr<br>290 295 300     | 914  |
| GTG GAC TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG<br>Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro<br>305 310 315     | 962  |
| GGG TAT CAC GCC TTT TAC TGC CAC GGA GAA TGC CCT TTT CCT CTG GCT<br>Gly Tyr His Ala Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala<br>320 325 330     | 1010 |
| GAT CAT CTG AAC TCC ACT AAT CAT GCC ATT GTT CAG ACG TTG GTC AAC<br>Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn<br>335 340 345 350 | 1058 |
| TCT GTT AAC TCT AAG ATT CCT AAG GCA TGC TGT GTC CCG ACA GAA CTC<br>Ser Val Asn Ser Lys Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu<br>355 360 365     | 1106 |
| AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG AAT GAA AAG GTT GTA TTA<br>Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu Asn Glu Lys Val Val Leu<br>370 375 380     | 1154 |
| AAG AAC TAT CAG GAT ATG GTT GTG GAG GGT TGT GGG TGT CGC<br>Lys Asn Tyr Gln Asp Met Val Val Glu Gly Cys Gly Cys Arg<br>385 390 395                     | 1196 |
| TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA                                                                                     | 1256 |
| AAAA                                                                                                                                                  | 1260 |

## (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 396 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

- 58 -

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val  
 1 5 10 15  
 Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys  
 20 25 30  
 Phe Ala Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu  
 35 40 45  
 Val Leu Ser Glu Phe Glu Leu Arg Leu Leu Ser Met Phe Gly Leu Lys  
 50 55 60  
 Gln Arg Pro Thr Pro Ser Arg Asp Ala Val Val Pro Pro Tyr Met Leu  
 65 70 75 80  
 Asp Leu Tyr Arg Arg His Ser Gly Gln Pro Gly Ser Pro Ala Pro Asp  
 85 90 95  
 His Arg Leu Glu Arg Ala Ala Ser Arg Ala Asn Thr Val Arg Ser Phe  
 100 105 110  
 His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr Ser Gly Lys Thr  
 115 120 125  
 Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu Glu Phe  
 130 135 140  
 Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln Met Gln Asp Ala  
 145 150 155 160  
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile  
 165 170 175  
 Ile Lys Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu  
 180 185 190  
 Asp Thr Arg Leu Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp  
 195 200 205  
 Val Thr Pro Ala Val Met Arg Trp Thr Ala Gln Gly His Ala Asn His  
 210 215 220  
 Gly Phe Val Val Glu Val Ala His Leu Glu Glu Lys Gln Gly Val Ser  
 225 230 235 240  
 Lys Arg His Val Arg Ile Ser Arg Ser Leu His Gln Asp Glu His Ser  
 245 250 255

- 59 -

Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly His Asp Gly Lys  
 260 265 270  
 Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His Lys Gln  
 275 280 285  
 Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp  
 290 295 300  
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr  
 305 310 315 320  
 His Ala Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His  
 325 330 335  
 Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val  
 340 345 350  
 Asn Ser Lys Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala  
 355 360 365  
 Ile Ser Met Leu Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys Asn  
 370 375 380  
 Tyr Gln Asp Met Val Val Glu Gly Cys Gly Cys Arg  
 385 390 395

## (2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 574 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: HOMO SAPIENS
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 1..327
  - (D) OTHER INFORMATION: /product= "MATURE hBMP3 (PARTIAL)"  
 /note= "THIS PARTIAL SEQUENCE OF THE MATURE HUMAN  
 BMP3 PROTEIN INCLUDES THE FIRST THREE CYSTEINES OF  
 THE CONSERVED 7 CYSTEINE SKELETON. SEE U.S. PAT.  
 NO. 5,011,691 FOR 102 C-TERMINAL SEQUENCE (CBMP3)."
- (ix) FEATURE:
  - (A) NAME/KEY: intron
  - (B) LOCATION: 328..574

- 60 -

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| CGA GCT TCT AAA ATA GAA TAC CAG TAT AAA AAG GAT GAG GTG TGG GAG   | 48  |
| Arg Ala Ser Lys Ile Glu Tyr Gln Tyr Lys Lys Asp Glu Val Trp Glu   |     |
| 1 5 10 15                                                         |     |
| GAG AGA AAG CCT TAC AAG ACC CTT CAG GGC TCA GGC CCT GAA AAG AGT   | 96  |
| Glu Arg Lys Pro Tyr Lys Thr Leu Gln Gly Ser Gly Pro Glu Lys Ser   |     |
| 20 25 30                                                          |     |
| AAG AAT AAA AAG AAA CAG AGA AAG GGG CCT CAT CGG AAG AGC CAG ACG   | 144 |
| Lys Asn Lys Lys Lys Gln Arg Lys Gly Pro His Arg Lys Ser Gln Thr   |     |
| 35 40 45                                                          |     |
| CTC CAA TTT GAT GAG CAG ACC CTG AAA AAG GCA AGG AGA AAG CAG TGG   | 192 |
| Leu Gln Phe Asp Glu Gln Thr Leu Lys Lys Ala Arg Arg Lys Gln Trp   |     |
| 50 55 60                                                          |     |
| ATT GAA CCT CGG AAT TGC GCC AGG AGA TAC CTC AAG GTA GAC TTT GCA   | 240 |
| Ile Glu Pro Arg Asn Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala   |     |
| 65 70 75 80                                                       |     |
| GAT ATT GGC TGG AGT GAA TGG ATT ATC TCC CCC AAG TCC TTT GAT GCC   | 288 |
| Asp Ile Gly Trp Ser Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala   |     |
| 85 90 95                                                          |     |
| TAT TAT TGC TCT GGA GCA TGC CAG TTC CCC ATG CCA AAG GTAGCCATTG    | 337 |
| Tyr Tyr Cys Ser Gly Ala Cys Gln Phe Pro Met Pro Lys               |     |
| 100 105                                                           |     |
| TTCTCTGTCC TGTACTTACT TCCTATTTCC ATTAGTAGAA AGACACATTG ACTAAGTTAG | 397 |
| TGTGCATATA GGGGGTTTGT GTAAGTGTTT GTGTTTCCAT TTGCAAAATC CATTGGGACC | 457 |
| CTTATTTACT ACATTCTAAA CCATAATAGG TAATATGGTT ATTCTTGTT TCTCTTAAT   | 517 |
| GGTTGTAAAA GTCATATGAA GTCAGTATTG GTATAAGAA GGATATGAGA AAAAAAA     | 574 |

## (2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 109 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

|                                                                 |
|-----------------------------------------------------------------|
| Arg Ala Ser Lys Ile Glu Tyr Gln Tyr Lys Lys Asp Glu Val Trp Glu |
| 1 5 10 15                                                       |

- 61 -

Glu Arg Lys Pro Tyr Lys Thr Leu Gln Gly Ser Gly Pro Glu Lys Ser  
                   20                  25                  30  
 Lys Asn Lys Lys Lys Gln Arg Lys Gly Pro His Arg Lys Ser Gln Thr  
                   35                  40                  45  
 Leu Gln Phe Asp Glu Gln Thr Leu Lys Lys Ala Arg Arg Lys Gln Trp  
                   50                  55                  60  
 Ile Glu Pro Arg Asn Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala  
                   65                  70                  75                  80  
 Asp Ile Gly Trp Ser Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala  
                   85                  90                  95  
 Tyr Tyr Cys Ser Gly Ala Cys Gln Phe Pro Met Pro Lys  
                   100                  105

## (2) INFORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1788 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS
- (F) TISSUE TYPE: HIPPOCAMPUS

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 403..1626
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"  
                           /product= "BMP2B"  
                           /evidence= EXPERIMENTAL  
                           /note= "BMP2B (CDNA)"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GAATTCGGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGCGC GGAGCCCGGC CCGGAAGCTA 60  
 GGTGAGTGTG GCATCCGAGC TGAGGGACGC GAGCCTGAGA CGCCGCTGCT GCTCCGGCTG 120  
 AGTATCTAGC TTGTCTCCCC GATGGGATTC CCGTCCAAGC TATCTCGAGC CTGCAGCGCC 180

- 62 -

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| ACAGTCCCCG GCCCTCGCCC AGGTTCACTG CAACCGTTCA GAGGTCCCCA GGAGCTGCTG | 240 |
| CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCCATTCC GTAGTGCCAT CCCGAGCAAC | 300 |
| GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG CTGTCAAGAA | 360 |
| TCATGGACTG TTATTATATG CTTTGTTTTT TGTCAGACA CC ATG ATT CCT GGT     | 414 |
| Met Ile Pro Gly                                                   |     |
| 1                                                                 |     |
| AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC   | 462 |
| Asn Arg Met Leu Met Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly   |     |
| 5 10 15 20                                                        |     |
| GCG AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCC   | 510 |
| Ala Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala   |     |
| 25 30 35                                                          |     |
| GAG ATT CAG GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG   | 558 |
| Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu   |     |
| 40 45 50                                                          |     |
| CTC CTG CGG GAC TTC GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC   | 606 |
| Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met Phe Gly Leu Arg   |     |
| 55 60 65                                                          |     |
| CGC CGC CCG CAG CCT AGC AAG AGT GCC GTC ATT CCG GAC TAC ATG CGG   | 654 |
| Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro Asp Tyr Met Arg   |     |
| 70 75 80                                                          |     |
| GAT CTT TAC CGG CTT CAG TCT GGG GAG GAG GAG GAA GAG CAG ATC CAC   | 702 |
| Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu Glu Glu Gln Ile His   |     |
| 85 90 95 100                                                      |     |
| AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG GCC AGC CGG GCC AAC ACC   | 750 |
| Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser Arg Ala Asn Thr   |     |
| 105 110 115                                                       |     |
| GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC CCA GGG ACC   | 798 |
| Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile Pro Gly Thr   |     |
| 120 125 130                                                       |     |
| AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC CCT   | 846 |
| Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile Pro   |     |
| 135 140 145                                                       |     |
| GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG CAG   | 894 |
| Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln   |     |
| 150 155 160                                                       |     |



- 63 -

|                                                                                                                                                       |      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| GTG GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT<br>Val Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile<br>165 170 175 180 | 942  |
| TAT GAG GTT ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC<br>Tyr Glu Val Met Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile<br>185 190 195     | 990  |
| ACA CGA CTA CTG GAC ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG<br>Thr Arg Leu Leu Asp Thr Arg Leu Val His His Asn Val Thr Arg Trp<br>200 205 210     | 1038 |
| GAA ACT TTT GAT GTG AGC CCT GCG GTC CTT CGC TGG ACC CGG GAG AAG<br>Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg Trp Thr Arg Glu Lys<br>215 220 225     | 1086 |
| CAG CCA AAC TAT GGG CTA GCC ATT GAG GTG ACT CAC CTC CAT CAG ACT<br>Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His Leu His Gln Thr<br>230 235 240     | 1134 |
| CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC CGA TCG TTA CCT CAA<br>Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg Ser Leu Pro Gln<br>245 250 255 260 | 1182 |
| GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC ACC TTT GGC<br>Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val Thr Phe Gly<br>265 270 275     | 1230 |
| CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC CGG AGG GCC AAG CGT<br>His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys Arg<br>280 285 290     | 1278 |
| AGC CCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC<br>Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys<br>295 300 305     | 1326 |
| CGG CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC<br>Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp<br>310 315 320     | 1374 |
| TGG ATT GTG GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC<br>Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp<br>325 330 335 340 | 1422 |
| TGC CCC TTT CCA CTG GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT<br>Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala Ile<br>345 350 355     | 1470 |
| GTG CAG ACC CTG GTC AAT TCT GTC AAT TCC AGT ATC CCC AAA GCC TGT<br>Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala Cys<br>360 365 370     | 1518 |

- 64 -

TGT GTG CCC ACT GAA CTG AGT GCC ATC TCC ATG CTG TAC CTG GAT GAG 1566  
 Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu  
 375 380 385  
 TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG ATG GTA GTA GAG GGA 1614  
 Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu Gly  
 390 395 400  
 TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG ATATACACAC 1666  
 Cys Gly Cys Arg  
 405  
 ACACACACAC ACACCACATA CACCACACAC ACACGTTCCC ATCCACTCAC CCACACACTA 1726  
 CACAGACTGC TTCCTTATAG CTGGACTTTT ATTTAAAAAA AAAAAAAAAA AAACCCGAAT 1786  
 TC 1788

## (2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 408 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Ile Pro Gly Asn Arg Met Leu Met Val Val Leu Leu Cys Gln Val  
 1 5 10 15  
 Leu Leu Gly Gly Ala Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys  
 20 25 30  
 Lys Lys Val Ala Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly  
 35 40 45  
 Gln Ser His Glu Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met  
 50 55 60  
 Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro  
 65 70 75 80  
 Asp Tyr Met Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu Glu  
 85 90 95  
 Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser  
 100 105 110  
 Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn  
 115 120 125

- 65 -

Ile Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu  
 130 135 140  
 Ser Ser Ile Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu  
 145 150 155 160  
 Phe Arg Glu Gln Val Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His  
 165 170 175  
 Arg Ile Asn Ile Tyr Glu Val Met Lys Pro Pro Ala Glu Val Val Pro  
 180 185 190  
 Gly His Leu Ile Thr Arg Leu Leu Asp Thr Arg Leu Val His His Asn  
 195 200 205  
 Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg Trp  
 210 215 220  
 Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His  
 225 230 235 240  
 Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg  
 245 250 255  
 Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu  
 260 265 270  
 Val Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg  
 275 280 285  
 Arg Ala Lys Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys  
 290 295 300  
 Asn Lys Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val  
 305 310 315 320  
 Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr  
 325 330 335  
 Cys His Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr  
 340 345 350  
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile  
 355 360 365  
 Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu  
 370 375 380  
 Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met  
 385 390 395 400

- 66 -

Val Val Glu Gly Cys Gly Cys Arg  
405

## (2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 102 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: HOMO SAPIENS
- (ix) FEATURE:
  - (A) NAME/KEY: Protein
  - (B) LOCATION: 1..102
  - (D) OTHER INFORMATION: /note= "BMP5"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |    |    |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|----|----|
| Cys | Lys | Lys | His | Glu | Leu | Tyr | Val | Ser | Phe | Arg | Asp | Leu | Gly | Trp | Gln | 1   | 5  | 10 | 15 |
| Asp | Trp | Ile | Ile | Ala | Pro | Glu | Gly | Tyr | Ala | Ala | Phe | Tyr | Cys | Asp | Gly | 20  | 25 | 30 |    |
| Glu | Cys | Ser | Phe | Pro | Leu | Asn | Ala | His | Met | Asn | Ala | Thr | Asn | His | Ala | 35  | 40 | 45 |    |
| Ile | Val | Gln | Thr | Leu | Val | His | Leu | Met | Phe | Pro | Asp | His | Val | Pro | Lys | 50  | 55 | 60 |    |
| Pro | Cys | Cys | Ala | Pro | Thr | Lys | Leu | Asn | Ala | Ile | Ser | Val | Leu | Tyr | Phe | 65  | 70 | 75 | 80 |
| Asp | Asp | Ser | Ser | Asn | Val | Ile | Leu | Lys | Lys | Tyr | Arg | Asn | Met | Val | Val | 85  | 90 | 95 |    |
| Arg | Ser | Cys | Gly | Cys | His |     |     |     |     |     |     |     |     |     |     | 100 |    |    |    |

## (2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 102 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- 67 -

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /note= "BMP6"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```

Cys Arg Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln
1 5 10 15
Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
 20 25 30
Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
 35 40 45
Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys
 50 55 60
Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
 65 70 75 80
Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Trp Met Val Val
 85 90 95
Arg Ala Cys Gly Cys His
 100

```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= OPX

/note= "WHEREIN XAA AT EACH POS'N IS INDEPENDENTLY  
 SELECTED FROM THE RESIDUES OCCURRING AT THE  
 CORRESPONDING POS'N IN THE C-TERMINAL SEQUENCE OF MOUSE  
 OR HUMAN OP1 OR OP2 (SEE SEQ. ID NOS. 1,8,10 AND 12.)"

- 68 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```

Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
1 5 10 15
Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
 20 25 30
Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
 35 40 45
Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
 50 55 60
Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
65 70 75 80
Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
 85 90 95
Xaa Ala Cys Gly Cys His
 100

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- 69 -

What is claimed is:

1. A method for promoting in vivo osseointegration of an implantable, prosthetic device, the method comprising the steps of:

providing on a surface of the prosthetic device substantially pure osteogenic protein, and

implanting the device in a mammal at a site wherein bone tissue and said surface are maintained at least partially in contact for a time sufficient to permit enhanced bone tissue growth between said tissue and said device.

2. In the method of repairing the skeletal system of a mammal comprising surgically implanting in contact with bone tissue a prosthetic device, and permitting the device and the bone tissue to integrate to form a weight bearing skeletal component, the improvement comprising:

providing substantially pure osteogenic protein on a surface of said device prior to its implantation thereby to promote enhanced bone tissue growth into said device and to improve the tensile strength of the junction between the bone and said device.

3. The method of claim 1 or 2 wherein said surface of said prosthetic device further comprises hydroxylapatite, collagen, homopolymers or copolymers of glycolic acid, lactic acid or butyric acid and derivatives thereof, tricalcium phosphate or other calcium phosphate, metal oxides or combinations thereof.

- 70 -

4. The method of claims 1 or 2 wherein the prosthetic device comprises a porous, metallic material.

5. The method of claim 1 or 2 wherein the osteogenic protein is an osteogenically active dimeric protein.

6. The method of claim 1 or 2 wherein the osteogenic protein is an osteogenically active dimeric protein produced by expression of recombinant DNA in a host cell, and comprises a pair of polypeptide chains, each of which has an amino acid sequence sufficiently duplicative of the sequence comprising residues 335 to 431 of Seq. ID No. 1 (OPS) such that said pair of polypeptide chains, when disulfide bonded to produce a dimeric species, has a conformation capable of inducing endochondral bone formation in association with said surface when implanted in a mammal.

7. The method of claim 1 or 2 wherein the osteogenic protein is an osteogenically active dimeric protein expressed from recombinant DNA in a host cell, characterized in that the protein comprises a pair of oxidized subunits disulfide bonded to produce a dimeric species, one of said subunits having an amino acid sequence encoded by a nucleic acid capable of hybridizing to a nucleic acid encoding OPS (residues 335 to 431 of Seq. ID No. 1) under stringent hybridization conditions, such that the disulfide bonded dimeric species comprising said subunit has a conformation capable of inducing endochondral bone formation in a mammal when disposed on the surface of said device.



- 71 -

8. The method of claim 1 or 2 wherein the osteogenic protein is an osteogenically active dimeric protein characterized in that one of the chains of said protein comprises an amino acid sequence sharing greater than 60% identity with an amino acid sequence comprising residues 335 to 431 of Seq. ID No. 1 (OPS).

9. The method of claim 8 wherein the osteogenic protein is an osteogenically active dimeric protein characterized in that the amino acid sequence of said chain of said protein comprises an amino acid sequence sharing greater than 65% identity with an amino acid sequence comprising OPS.

10. The method of claim 9 wherein the osteogenic protein is an osteogenically active dimeric protein characterized in that the amino acid sequence of said chain of said protein comprises residues 335-431 of Seq. ID No. 1 (OPS).

11. The method of claim 9 wherein the osteogenic protein is an osteogenically active dimeric protein which is a homodimer, wherein both chains comprise the amino acid sequence of OPS (residues 335-431 of Seq. ID No.1.)

12. The method of claim 11 wherein both chains of said osteogenically active dimeric protein comprise the amino acid sequence of residues 293-431 of Seq. ID No. 1 (OP1-18Ser.)

13. An improved prosthetic device for repairing mammalian skeletal defects, injuries, or anomalies comprising a rigid prosthetic implant having a porous or non-porous surface region for implantation adjacent bone tissue, wherein the improvement comprises:

- 72 -

substantially pure osteogenically active osteogenic protein disposed on said surface region in an amount sufficient to promote enhanced bone tissue growth into said surface.

14. The device of claim 13 wherein said surface of said prosthetic device further comprises hydroxylapatite.

15. The device claim 13 wherein the osteogenic protein is an osteogenically active dimeric protein.

16. The device of claim 13 wherein the osteogenic protein is an osteogenically active dimeric protein produced by expression of recombinant DNA in a host cell, and comprises a pair of polypeptide chains, each of which has an amino acid sequence sufficiently duplicative of the sequence comprising residues 335-431 of Seq. ID No.1 (OPS), such that said pair of polypeptide chains, when disulfide bonded to produce a dimeric species, has a conformation capable of inducing endochondral bone formation in association with said surface when implanted in a mammal.

17. The device of claim 13 wherein the osteogenic protein is an osteogenically active dimeric protein characterized in that the protein comprises a pair of oxidized subunits disulfide bonded to produce a dimeric species, one of said subunits having an amino acid sequence encoded by a nucleic acid capable of hybridizing to a nucleic acid encoding OPS (residues 335-431 of Seq. ID No. 1), such that the disulfide bonded dimeric species comprising said subunit has a conformation capable of inducing endochondral bone formation in a mammal when disposed on the surface of said device.

- 73 -

18. The device of claim 13 wherein the osteogenic protein is an osteogenically active dimeric protein characterized in that one of the chains of said protein comprises an amino acid sequence sharing greater than 65% identity with an amino acid sequence comprising OPS (residues 335 to 431 of Seq. ID No. 1).

19. The device of claim 18 wherein the osteogenic protein is an osteogenically active dimeric protein characterized in that the amino acid sequence of said chain of said protein comprises an amino acid sequence sharing greater than 65% identity with an amino acid sequence comprising OPS (residues 335-431 of Seq. ID No. 1).

20. The device of claim 19 wherein the osteogenic protein is an osteogenically active dimeric protein characterized in that the amino acid sequence of said chain of said protein comprises residues 335-431 of Seq. ID No. 1 (OPS).

21. The device of claim 19 wherein the osteogenic protein is an osteogenically active dimeric protein which is a homodimer, wherein both chains comprise the amino acid sequence of OPS (residues 335-431 of Seq. ID No. 1).

22. The device of claim 21 wherein wherein both chains of said osteogenically active dimeric protein comprise the amino acid sequence of residues 293-431 of Seq. ID No.1 (OP1-18Ser.)

23. The device of claim 13 wherein the prosthesis comprises a porous metallic material.

- 74 -

24. The device of claim 13 wherein the prosthesis comprises a contoured implantable portion for insertion into an orifice having plural indentations transverse to its longitudinal axis.

25. The device of claim 24 comprising a dental implant.

26. A method for promoting in vivo osseointegration of a prosthetic device into an orifice of a bone, comprising the steps of:

providing a prosthetic device having a contoured implantable portion for insertion into said orifice, said contoured portion having plural indentations transverse to its longitudinal axis, and

implanting into the orifice the contoured portion of the prosthetic device and a bone growth composition comprising a substantially pure osteogenic protein combined with a matrix material which induces bone growth in said indentations, osseointegration between the bone and the prosthetic device, and osseointegration of new bone induced by said composition and said bone.

27. The method of claim 26 wherein the contoured portion comprises a porous metallic material.

28. The method of claim 27 wherein the osteogenic protein enhances bone ingrowth into said pores.

29. A device for promoting in vivo osseointegration of a prosthesis into an orifice of a bone, comprising

a rigid prosthetic implant having a contoured portion for insertion into said orifice, said contoured portion having plural indentations transverse to its longitudinal axis, and

a bone growth composition comprising a substantially pure osteogenic protein combined with a matrix material which induces bone growth in said indentations, osseointegration between the bone and the prosthetic implant and osseointegration of new bone induced by said composition and said bone.

30. The device of claim 29 wherein the contoured portion comprises a porous metallic material.

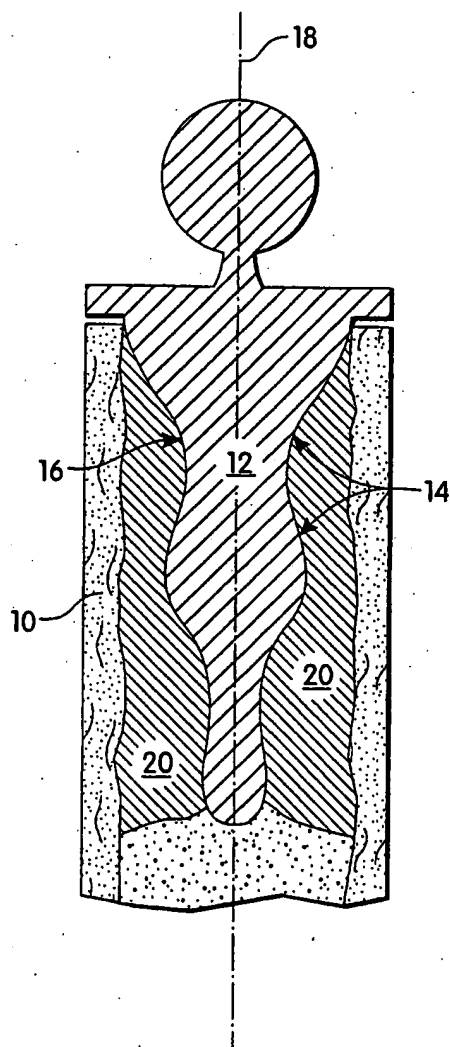
31. The device of claim 30 wherein the osteogenic protein enhances bone ingrowth into said pores.

32. The device of claim 29 wherein said matrix material is selected from the group consisting of hydroxylapatite, collagen, polymers or copolymers of glycolic acid, lactic acid or butyric acid, tricalcium phosphate or other calcium phosphates, metal oxides, demineralized guanidine extracted bone and combinations thereof.

33. The device of claim 29 comprising a dental implant.

34. The device of claim 29 wherein the osteogenic protein is an osteogenically active dimeric protein produced by expression of recombinant DNA in a host cell, and comprises a pair of polypeptide chains, each of which has an amino acid sequence sufficiently duplicative of the sequence comprising residues 335 to 431 of Seq. ID No. 1 (OPS) such that said pair of polypeptide chains, when disulfide bonded to produce a dimeric species, has a conformation capable of inducing endochondral bone formation in association with said contoured portion of said prosthesis when implanted in a mammal.

1/1



## INTERNATIONAL SEARCH REPORT

PCT/US 93/05446

International Application No

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                            |                                                     |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------|
| <b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                            |                                                     |
| According to International Patent Classification (IPC) or to both National Classification and IPC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                            |                                                     |
| Int.Cl. 5 A61L27/00; A61K37/02; A61K6/00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                            |                                                     |
| <b>II. FIELDS SEARCHED</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                            |                                                     |
| Minimum Documentation Searched <sup>7</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                            |                                                     |
| Classification System                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Classification Symbols                                                                                                                     |                                                     |
| Int.Cl. 5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | A61L ; A61K ; C07K                                                                                                                         |                                                     |
| Documentation Searched other than Minimum Documentation<br>to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                            |                                                     |
| <b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                            |                                                     |
| Category <sup>10</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>                             | Relevant to Claim No. <sup>13</sup>                 |
| X                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | WO,A,8 800 205 (GENETICS INSTITUTE)<br>14 January 1988<br>cited in the application                                                         | 13,14,23                                            |
| Y                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | see page 9, line 1 - line 2; claims 1,2,7<br><br>see abstract<br>---                                                                       | 15-22,<br>24,25,<br>29-34                           |
| X                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | EP,A,0 361 896 (COLLAGEN CORPORATION)<br>4 April 1990                                                                                      | 13,14,23                                            |
| Y                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | see column 5, line 28 - line 53<br>see column 7, line 19 - line 26; claims<br>1,3-7,16,19<br>---                                           | 30-32                                               |
| X                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | EP,A,0 182 483 (COLLAGEN CORPORATION)<br>28 May 1986<br>see page 13, line 12 - line 19<br>see page 5, line 1 - line 3; claims 1,3,7<br>--- | 13,14,23                                            |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | -/--                                                                                                                                       |                                                     |
| <sup>10</sup> Special categories of cited documents :<br><sup>"A"</sup> document defining the general state of the art which is not considered to be of particular relevance<br><sup>"E"</sup> earlier document but published on or after the international filing date<br><sup>"L"</sup> document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)<br><sup>"O"</sup> document referring to an oral disclosure, use, exhibition or other means<br><sup>"P"</sup> document published prior to the international filing date but later than the priority date claimed<br><sup>"T"</sup> later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention<br><sup>"X"</sup> document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step<br><sup>"Y"</sup> document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.<br><sup>"&amp;"</sup> document member of the same patent family |                                                                                                                                            |                                                     |
| <b>IV. CERTIFICATION</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                            |                                                     |
| Date of the Actual Completion of the International Search                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                            | Date of Mailing of this International Search Report |
| 14 OCTOBER 1993                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                            | 28. 10. 93                                          |
| International Searching Authority<br>EUROPEAN PATENT OFFICE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                            | Signature of Authorized Officer<br>PELTRE CHR.      |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) |                                                                                                                                                  |                       |
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| Category °                                                                 | Citation of Document, with indication, where appropriate, of the relevant passages                                                               | Relevant to Claim No. |
| Y                                                                          | WO,A,9 105 802 (CREATIVE BIOMOLECULES)<br>2 May 1991<br>cited in the application<br>see page 69 - page 70<br>see page 3, line 1 - line 3<br>---- | 15-22, 34             |
| Y                                                                          | EP,A,0 106 946 (SULZER)<br>2 May 1984<br>see figure 3<br>----                                                                                    | 24, 29                |
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| A                                                                          | EP,A,0 470 305 (OSTEOTECH)<br>12 February 1992<br>----                                                                                           |                       |
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/05446

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 1-12, 26-28 are directed to a method of treatment of  
(diagnostic method practised on) the human/animal body the search has been  
carried out and based on the alleged effects of the compound.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such  
an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9305446  
SA 76365

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
The members are as contained in the European Patent Office EDP file on  
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14/10/93

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US 9305446  
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14/10/93

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